ELSEVIER

PII: S0261-2194(97)00078-1

Crop Protection Vol. 17, No. 1, pp. 35–39, 1998
Published by Elsevier Science Ltd
Printed in Great Britain
0261-2194/98 $19.00 + 0.00

Field tests of denatonium benzoate to reduce seedling damage by pocket gophers (*Thomomys talpoides* Rich.)

Gary W. Witmer*‡, Michael J. Pipas* and John C.Bucher†

**USDA APHIS National Wildlife Research Center, Dept. of Natural Resource Sciences, Washington State University, Pullman, WA 99164-6410, USA and †Department of Forest Resources, University of Idaho, Moscow, ID 83843, USA*

The repellency of a bitter compound, denatonium benzoate, to reduce pocket gopher damage to conifer seedlings was tested in two independent field trials in Oregon and Idaho. In the Oregon trial (1992 to 1993), treatments included a denatonium benzoate tablet placed in-ground with the seedling roots; a tablet plus denatonium benzoate foliar spray applied to both roots and foliage; and no chemical application (i.e. control). No significant difference between treatments was noted for ponderosa pine (*Pinus ponderosa* Dougl.) or Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) seedlings suffering gopher-related mortality. Non-animal mortality (58.2% of all seedlings) accounted for a greater loss of seedlings than gophers (38.2%). Composite foliage and composite soil samples collected from the treatment plots were all negative for the presence of denatonium benzoate. In the Idaho trial (1993 to 1994), similar treatments (but with an additional spray-only treatment) were used on ponderosa pine seedlings. There was no significant difference in gopher-related mortality levels between treatments. A large portion (72.5%) of all seedlings was destroyed by gophers. It appears that the bitter compound, denatonium benzoate, as evaluated in these trials, is not an effective gopher repellent. Published by Elsevier Science Ltd

Keywords: damage; denatonium benzoate; pocket gopher; repellent; *Thomomys*

Pocket gophers have historically played a significant role in reforestation efforts in the Pacific Northwest by causing widespread and severe damage to tree seedlings (Marsh and Steele, 1992). This damage includes stem, branch and root clipping of tree seedlings and girdling of larger seedlings and saplings (Teipner *et al.*, 1983). Historically, toxic baits and kill traps have been used to reduce gopher populations and allow reforestation (Case and Jasch, 1994; Witmer *et al.*, 1995). The nature and extent of this problem are sources of constant concern to resource managers, who are always searching for new solutions to an old problem.

The use of chemical repellents is a promising control technique that is currently in the forefront of research approaches. Current vertebrate repellents are intended to protect above-ground plant parts by a noxious odor (area repellent) or taste (contact repellent; Craven and Hygnstrom, 1994). As gophers can damage plants both above and below ground, a systemic repellent (one taken up by the plant and distributed throughout its tissues) could provide more complete protection. Considerable effort has been made to develop selenium as a systemic repellent (Allan *et al.*, 1983), but no products have been registered in the USA, in part because of potential toxicity. Various formulations of denatonium benzoate, a very bitter compound, have been developed as a bittering additive to various household products, to deter nail-biting and thumb-sucking, and as contact animal repellents (Kaukeinen and Buckle, 1992). Denatonium benzoate could, in theory, provide foliar protection to seedlings when applied as a spray, and systemic protection in tablet form when placed in-ground with newly planted seedlings. The mode of action is reported to be gustatory, with the chemical imparting a very bitter taste to treated seedlings (Payne, 1988). Most data on the effectiveness of denatonium benzoate as an animal repellent come from unpublished sources. Denatonium benzoate is attractive because of its relatively low toxicity; Randolph (1980) reported an oral LD_{50} of 612–820 mg/kg for adult rats. Additionally, because of its extreme bitterness, it is unlikely that an animal would consume a lethal dose. We conducted two field trials to evaluate the repellency of denatonium benzoate to reduce pocket gopher damage to conifer seedlings on reforestation units of the Mt. Hood National Forest, Oregon, and a year later on the Clearwater National Forest, Idaho.

‡To whom correspondence should be addressed.

Materials and methods

Oregon field trial

The Oregon field trial was conducted May 1992–1993 on a clearcut unit of the Mt. Hood National Forest, Wasco County, Oregon. The unit was replanted in Spring, 1990. The study site was in the ponderosa pine forest zone (Franklin and Dyrness, 1973) and was characterized by a low forest productivity level (classes IV–V). The pumice soils were relatively shallow with low water holding capacity. Annual precipitation was 76 cm per year, mostly as snow. Summers were hot and dry. Elevation was 1155 m above sea level. The 5–15% slope faced southeast.

We used a split-plot experimental design with randomized plots (Ott, 1993). Thirty plots (1920 seedlings) were established, each with eight rows of eight seedlings with 2.4 m spacing. Ponderosa pine and Douglas fir seedlings (1/0 nursery stock) were planted alternately within rows. Each plot was centered over an active gopher burrow system to ensure equal exposure of both species to a foraging gopher. Active systems were identified by the open hole survey method (Barnes *et al.*, 1970).

Three treatments were randomly applied each of ten plots in May 1992: (1) placement of a 1.5 g tablet containing 210 mg denatonium benzoate (14% active ingredient) in the ground with the seedling at time of planting; (2) same application as above with the addition of a foliar spray of denatonium benzoate (2% active ingredient in a latex carrier, applied until free flowing) applied to the seedling roots before planting and the foliage after planting; (3) no chemical application (i.e. control). Concentrations used were selected because they are commercially available tablets and spray (Ani-pel®). Reference to trade names does not imply US government endorsement of commercial products or exclusion of a similar product with equal or better effectiveness.

Seedlings were checked monthly through 6 months and then at 12 months post-planting (after winter). Each seedling was measured and evaluated for condition (injured, healthy) or fate (dead or missing). Mortality in this study was classified as either non-animal (primarily from drought) or gopher-related. Sharp, oblique cuts of stems or branches along with fresh gopher sign were indicative of gopher-caused damage or mortality. Missing seedlings were recorded as gopher mortalities if there was fresh gopher sign (mounds, burrows) nearby and no trace of the seedling could be found.

Foliage samples were taken from a majority of the control and tablet-treated plots, 3 months post-planting. A few new growth needles from the tips of the upper laterals were chosen from each sampled plant. Samples of needles were pooled by species (Douglas fir or ponderosa pine) and treatment (control or tablet-treated), yielding four composite samples. Samples were freeze dried at −60°C. Composite soil samples were collected from both the tablet and tablet/spray plots. These samples were taken from numerous plots 6 in deep near the roots and pooled for each treatment. Samples were submitted to Monell Chemical Senses Center in Philadelphia, PA, for assay of denatonium benzoate using high performance liquid chromatography (HPLC). At 210 nm ultraviolet wavelength, denatonium benzoate standards of 0.5 ppm were easily detected.

The percentages of seedling mortalities were compared by treatment within 1, 6, and 12 month periods and cumulatively over time. We used a univariate form of repeated measures analysis of variance (ANOVA, SAS Institute Inc., 1985) followed by Duncan's new multiple range test (Ott, 1993). All tests were conducted at the $P = 0.05$ significance level. An arc-sine transformation was performed on the data and a sphericity test applied to determine that the F-tests were exact and consequently met the assumption of Type H covariance (SAS Institute Inc., 1985).

Idaho field trial

The Idaho field trial was conducted from May 1993–1994 on three clearcut units of the Clearwater National Forest, Latah County, Idaho. The units were in western hemlock/queencup beadlily (*Tsuga heterophylla* (Raf.) Sarg./*Clintonia uniflora* (Schult.) Kunth.) habitat types (Cooper *et al.*, 1991), were 850 m above sea level, and had heavy pocket gopher activity. The soil type for units 1 and 2 was Eutric glossoboralf (ashy phase), medial to fine loamy, mixed, frigid, overlying residuum and colluvium derived from metasedimentary rock. The soil type for unit 3 was Typic Vitrandepts, medial to loamy-skeletal, mixed, frigid; and Andic Dystrochrepts, loamy-skeletal, mixed and frigid.

Unit 1 had a 29% slope and faced northwest. It was harvested in 1989, planted in 1990, and interplanted in 1992 with western white pine (*Pinus monticola* Dougl.), Douglas fir, western larch (*Larix occidentalis* Nutt.), and ponderosa pine seedlings. Unit 2 was harvested in 1989, planted in 1990 and interplanted, as above, in 1992. Natural grand fir (*Abies grandis* (Dougl.) Forbes) seedling regeneration was present in the area. The unit had a 16% slope and faced northeast. Unit 3 was harvested in 1984 and planted in 1985 with lodgepole pine (*Pinus contorta* Dougl.) and western white pine. It had an 8% slope and faced southwest.

Each of the three plots was planted with 120 containerized pondersosa pine seedlings with 2 m spacing. Each plot of 120 seedlings was planted with 30 untreated (control) seedlings, 30 treated with denatonium benzoate spray only, 30 treated with denatonium benzoate tablets only, and 30 treated with both tablets and above-ground spray. The treatment materials were the same commercially available materials used in the Oregon trial and were applied in the same way. The treated seedlings were distributed systematically throughout the plots to ensure equal exposure to gophers.

For the first 10 weeks, seedlings were inspected for gopher or other mortality. Two categories were used in addition to gopher-caused mortality: other animal (hare, *Lepus* spp. Linn.; ungulates, deer, *Odocoileus* spp. Rafin., and elk, *Cervus elaphus* Linn.; and cattle)

and non-animal (primarily from drought). Seedling mortality analyses were divided into three periods: May–August 1993, September–October 1993, and November 1993–April 1994. ANOVA was conducted to test for differences between treatments at the $P = 0.05$ significance level.

Results

Oregon field trial

There were no significant differences among treatments ($P = 0.15$; *Table 1*) in seedling mortality attributed to gophers. However, a significantly greater proportion of ponderosa pine (43.9%, 420 of 957 seedlings) than Douglas fir (32.5%, 310 of 955 seedlings) were lost to gophers ($P = 0.0001$). No treatment by species interaction was noted ($P = 0.39$). On a time scale, differences in damage levels were highly significant between months ($P = 0.0001$), ranging from 4.1% 1 month following planting to 38.2% 12 months post-planting, showing a substantial cumulative effect by gophers. In addition, a significant time by treatment interaction was noted ($P = 0.0028$), mortality values within treatments routinely increasing by a factor of 7–12 between months 1 and 12.

Non-animal mortality, primarily from drought, was a much more serious threat than gophers were to seedlings (*Table 1*). Of the 1912 seedlings, 58.2% (1112) were classed as non-animal mortalities, whereas 38.2% (730) were classed as gopher-related mortalities. Treatment did not confer a survival edge to seedlings (i.e. no treatment difference, $P = 0.08$). Significantly more Douglas fir (62.3%, 595 of 955) than ponderosa pine (54%, 517 of 957) were lost to non-animal causes ($P = 0.01$). A highly significant time effect was noted ($P = 0.0001$). Mean damage at 1 month was significantly lower than that at months 6 and 12, according to Duncan's multiple range test. There was a significant time by treatment interaction. Damage levels within treatments between months 1 and 6 increased dramatically, but increased only slightly between months 6 and 12.

Total mortality, as defined by removal of the species variable, showed the same general patterns for both non-animal and gopher-related mortality (*Table 1*). For both gopher-related mortality and non-animal mortality sources, treatment effects were not significant ($P = 0.15$ and $P = 0.08$ respectively). Mortality differences by time were highly significant ($P = 0.0001$) for both sources of mortality. A treatment by time interaction was noted only for total gopher-related mortality ($P = 0.04$). However, a treatment within time effect was indicated by the Duncan grouping at the 12 month level for both total gopher-related and total non-animal mortality sources. In the case of total gopher mortality, tablet/spray-treated seedlings endured significantly less mortality than control seedlings, but for total non-animal mortality at the 12 month time level this pattern was reversed.

All four composite foliage samples were negative for denatonium benzoate (0 ppm). The two composite soil samples from tablet and tablet/spray plots were also negative for the presence of this chemical (0 ppm).

Idaho field trial

No significant differences in gopher-related mortality levels were found between treatments ($P = 0.83$) or plots ($P = 0.75$) (*Table 1*). A total of 301 ponderosa pine seedlings (83.6%) of the original 360 died during the 1 year study. A total of 261 seedlings (72.5%) were destroyed by gophers. Only 40 seedlings (11.1%) of the 360 seedlings were destroyed by other animals or non-animal causes, representing a minor proportion of total seedling mortality (*Table 1*).

From May to August, gophers accounted for 135 (90%) of 150 seedling mortalities. During this period, 41.7% of all seedlings planted were lost. In the second period (August–October), 67 (31.9%) of 210 remaining seedlings were lost, with gophers again responsible for a large, but smaller proportion (73.1%) of seedlings lost. In the third period (October–April), 84 (58.7%) seedlings died of the 143 remaining. Gophers caused 77 (53.8%) of these mortalities. Gopher-related mortality occurred throughout the field trial, but in declining proportions of remaining seedlings.

Table 1. Average seedling mortality by source for field trials with denatonium benzoate in Oregon (1912 Douglas fir and ponderosa pine seedlings) in 1992–1993 and Idaho (360 ponderosa pine seedlings) in 1993–1994

Mortality source	Number of seedlings	Average annual mortality (%) by treatment[a]				Total mortality by source
		Control	Tablet	Tablet and spray[b]	Spray[c]	
Oregon						
Gopher	730	24.3	22.5	18.6	—	38.2
Non-animal	1112	35.3	39.2	43.5	—	58.2
Idaho						
Gopher	261	22.3	20.0	23.0	21.7	72.5
Other animal	32	5.8	8.3	8.3	4.2	8.9
Non-animal	8	1.7	1.7	0.8	2.5	2.2

[a]Numbers in a row followed by the same letter are not different according to Duncan's multiple range test, $P = 0.05$. No letters indicate no significant differences.
[b]In the Oregon trial, both above- and below-ground seedling parts were sprayed; in the Idaho trial, only above-ground seedling parts were sprayed.
[c]A spray-only treatment was not included in the Oregon trial.

Discussion

In these field trials, denatonium benzoate did not offer seedlings significant protection against foraging pocket gophers. Results in the Oregon trial may have been confounded by the disproportionately high level of non-animal (primarily from drought) mortality (58.2% of all seedlings) that may have masked potential real differences in repellent efficacy. However, non-animal mortality levels were very low (2.2%) in the Idaho field trial.

Despite differences in mortality between tree species in the Oregon trial, no consistent pattern emerged. A significantly larger proportion of ponderosa pine (43.9%) than Douglas fir (32.5%) was lost to pocket gophers, but this pattern was reversed for non-animal mortality (54% versus 62.3%). However, total mortality after 12 months did not differ substantially between species (ponderosa pine, 97.9%; Douglas fir, 94.8%). The Idaho field trial used only ponderosa pine seedlings, but these were largely (72.5%) destroyed by gophers.

Seedling mortality did have a highly significant time component in the Oregon trial that was evident in all analyses. Gopher-related mortality increased significantly across all three periods of time, whereas non-animal mortality showed a significant increase only between months 1 and 6. This latter scenario is probably attributable to onset of weather conditions between months 6 and 12 more amenable to growth and survival (i.e. cooler temperatures and more soil moisture). On the other hand, increased gopher-related mortality over the entire time period could have been induced by the onset of winter that increased foraging opportunities for gophers burrowing through the snow and a decline in other sources of forage. In the Idaho trial, gopher-related mortality was substantial in all time periods ($> 50\%$), but greatest (90%) in the initial 3 months.

Negative results for denatonium benzoate for both the composite foliage and composite soil assays in the Oregon trial prompted a closer look at the fate of the chemical on the plots. On a subsequent field visit (17 November 1992), a few dead seedlings in the tablet and tablet/spray plots were unearthed to search for remnants of the tablets. In a majority of the cases, large fragments of the tablets still remained. Most likely, low soil moisture through the first six months of the study inhibited tablet breakdown. However, outdoor nursery trials with Douglas fir seedlings in Olympia, WA, under much more favorable moisture regimes and using the same 1.5 g tablets gave variable rates of uptake of denatonium benzoate; concentrations in foliage samples ($n = 7$) ranged from 0.0–21.6 ppm and averaged 7.9 ppm (unpublished data).

In the Oregon trial, the most important seedling mortality factor appeared to be lack of precipitation (and perhaps poor planting stock) that resulted in high levels of non-animal mortality. Seedlings were disadvantaged from the start. The first day of planting was typical of the summer in general: hot (temperatures about 37.8°C) dry weather conditions. None of the seedlings were artificially watered at any point in that trial. The first 6 months following planting were characterized by very low precipitation (< 15 cm total). The first 3 months post-planting, the most critical for survival of seedlings, received a total of only 2 cm of precipitation. This, combined with high temperatures, drastically reduced soil moisture levels and inhibited tablet breakdown. Consequently, assimilation of denatonium benzoate by seedlings was seriously impaired.

In the Idaho trial, similar results were achieved despite more favorable moisture conditions and much lower levels of non-animal mortality. It is important to note that even direct foliar spraying (and root spraying in the Oregon trial) did not significantly reduce gopher damage to seedlings at the concentration used (2% active ingredient). Consequently, denatonium benzoate may not be an effective repellent of gophers unless higher dosages can be achieved within economic constraints. Additional research would be needed to test this possibility.

References

Allan, G. G., Gustafson, D. L., Mikels, R. A., Miller, J. M. and Neogi, S. (1983) Reduction of deer browsing of Douglas fir seedlings by quadrivalent selenium. *For. Ecology Manage.* **7**, 163–181

Barnes, V. G. Jr., Martin, P. and Tistjan, H. P. (1970) Pocket gopher control on ponderosa pine plantations. *J. For.* **68**, 433–435

Case, R. M. and Jasch, B. A. (1994) Pocket gophers. In *Prevention and Control of Wildlife Damage*, eds S. Hygnstrom, R. Timm, and G. Larsen, pp. B-17–B-29. University of Nebraska Press, Lincoln, NE.

Cooper, S. V., Neiman, K. E. and Roberts, D. W. (1991) Forest Habitat Types of Northern Idaho: A Second Approximation. United States Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Gen. Tech. Rep. INT-GTR-236, Ogden, UT, 143 pp.

Craven, S. R. and Hygnstrom, S. E. (1994) Deer. In *Prevention and Control of Wildlife Damage*, eds S. Hygnstrom, R. Timm and G. Larsen, pp. D-25–D-40. University of Nebraska Press, Lincoln, NE.

Franklin, J. F. and Dyrness, C. T. (1973) Natural Vegetation of Oregon and Washington. United States Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, GTR-PNW-GTR-8, Portland, OR, 417 pp.

Kaukeinen, D. E. and Buckle, A. P. (1992) Evaluations of aversive agents to increase the selectivity of rodenticides, with emphasis of denatonium benzoate bittering agent. *Proc. Vertebr. Pest Conf.* **15**, 192–198

Marsh, R. E. and Steele, R. W. (1992) Pocket gophers. In Silvicultural Approaches to Animal Damage in Pacific Northwest Forests, ed. H. C. Black, pp. 205–230. United States Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, Gen. Tech. Rep. PNW-GTR-287, Portland, OR.

Ott, R. L. (1993) *An Introduction to Statistical Methods and Data Analysis*, 4th edition. Wadsworth Publishing Company, Belmont, CA, 1038 pp.

Payne, H. A. S. (1988) Bitrex — a bitter solution to safety. *Chemistry and Industry* **22**, 721–723

Randolph, W.P. (1980) Nail biting and thumbsucking deterrent drug products for over-the-counter human use: establishment of a monograph. *Federal Register* **405**, 69122–69128

SAS Institute Inc. (1985) *SAS User's Guide: Statistics*. Version 5. SAS Institute Inc., Cary, NC, 956 pp.

Teipner, C. L., Garton, E. O. and Nelson, L., Jr. (1983) Pocket Gophers in Forest Ecosystems. United States Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Gen. Tech. Rep. INT-GTR-154, Ogden, UT, 53 pp.

Witmer, G. W., Matschke, G. H. and Campbell, D. L. (1995) Field trials of pocket gopher control with cholecalciferol. *Crop Protection* **14**, 307–309

Received 21 April 1997
Revised 21 July 1997
Accepted 15 September 1997